Evaluation of m-TEC vs. Colilert Quanti Tray 2000

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City of Anderson's Water Pollution Control Department (WPC) is renewing its National Pollutant Discharge Elimination System (NPDES) permit with the Indiana Department of Environmental Management. Our current permit requires the POTW to report fecal coliforms (MPN) on the treated effluent to the receiving stream from April 1, through October 31. The new permit will require the reporting of *Escherichia coli (E.coli).* This change is due to the U.S. Environmental Protection Agency (EPA) attention has now been centered on *E.coli,* because it is felt that it is a more reliable indicator of fecal contamination. The EPA recommends, (it is not EPA approved) the m-TEC for the method of identification and enumeration of *E.coli.* Anderson's' WPC Laboratory staff has had previous experience with the m-TEC Method. They found the method to be time consuming and restrictive, due to the 2-hour delay in incubation time and the 15-minute time window to read the plates. Reading the plates tended to be subjective in distinguishing the yellow to yellow-brown *E.coli* colonies. Another problem was that the colonies continued to change to purple, thus giving false negative results. The staff had previously used a product called Colilert (produced by IDEXX Laboratories, Westbrook, Maine) for analyzing drinking water for *E.coli* and occasionally Colilert Quaint-Tray on ponds for *E.coli* and total coliforms. The Quanti-Tray combines speed, ease and accuracy and is based on the Standard Methods Most Probable Number (MPN) statistical model. There is no media or indicator preparation, less than 3 minutes hands on time, colony counting and heterotrophic interference are eliminated. Results are obtained in 24 hours for *E.coli* and total coliforms. There were no problems distinguishing positive (fluorescing] for *E.coli* results. After lengthy discussion with IDEM, it was determined to do a study to comparing m-TEC and Colilert Quanti Tray (manufactured by Access Analytical Laboratories) methods. We were asked provide the following information:

- ➢ detection and enumeration of at least 50 split samples.
- ➢include statistics that show the relationship between the two methods, i.e. a regression analysis
- ➢ explain the relationship between Colony Forming Units (CPU) and Most Probable Number (MPN)
- ➢state the degree of confidence that can be measured about the inferences
- ➢verify a portion of the tests and state the false positive and false negative results.

We also looked at the time and attention associated with media preparation, set-up, initial analysis, clean up and interpreting the final results.

DEVELOPMENT, FUNCTION and PROCEDURE

m-TEC

Background:

The m-TEC method utilizes 100 mL of filtered sample that is incubated at 35 ± 0.5°C for 2 hours for the recovery of *E.coli* that may have been injured or stressed during disinfection or filtration. It is then incubated 44.5 ± 0.5°C for an elevated temperature causing metabolic production of organic acids from lactose fermentation. These acids cause a pH change thus reacting with the indicators, Bromcresol purple and Bromphenol red, to exhibit a color changes. Dark purple colonies, confluent growth, heterotrophic organisms, make it difficult to differentiate *E.coli* with confidence.

Apparatus:

A. Reagents:

m-TEC agar-prepared according to manufactures instructions. Autoclave. Pour prepared media into sterile 9x50mm petri dishes {~4mL per dish). Leave lids tilted. When solidified, store at 4 C for 1 month.

Urease-Mix well 2.0g urea, 0.0lg phenol red and 100 mL of deionized water. Adjust pH to 3.5±0.5 with .IN HC1, drop wise. Good for 7 days

Buffered dilution water-*Phosphate buffer*: Weigh 34g KH_2PO_4 and dilute to ~400mL in a 500mL volumetric flask, with distilled water. Adjust to a pH of 7.2 with 1N NaOH and bring to volume. *MgCl*: Weigh 38g MgCl and dilute to 500mL volumetric flask, with distilled water. phosphate monobasic monohydrate, 5.0g sodium phosphate dibasic and 17.0g Sodium chloride into weighing boats. Transfer contents, rinsing the weighing boats, to a 2-liter volumetric flask. Dilute to 2 liters. Pour into autoclaveable bottles and autoclave for 15 minutes.

95% Methanol

Colilert

Background:

Colilert was originally developed for use in clinical laboratories to enumerate and identify bacteria in urine. It was then adapted to identify total coliforms and *E.coli* in the analysis of drinking water. As Total coliform and *E.coli* bacteria metabolize the nutrient indicators in Colilert they cause two distinct reactions. The two nutrient indicators are: (1) 4-methylum-belliferyl-β−D-glucuronide that changes from clear to yellow for Total coliform and (2) o-nitrophenyl- β-D-galacto-pyranoside that will emit a definite blue fluorescence using a long-wavelength 366 *nm* ultraviolet light for *E.coli*.

Apparatus:

A. Reagents:

Colilert Media-Snap Pack [IDEXX Laboratories]-blister packs of reagent, for 100 mL water samples. Shelf life: up to 12 months. Store at 4-30°C.

Color Comparator [IDEXX Laboratories] is a liquid color reference reagent used to distinguish a minimum positive from a negative test results at 24 hours.

m-TEC

B. Equipment:

Filter Apparatus
Pipets
Sample bottles- sterilized with sodium thiosulfate added
Petri Dishes 9x50mm
Incubator (35 ± 0.5°C]
Water bath (44 ± 0.5°C]
Autoclave
Baggies
Forceps
Bunsen Burner
Weights to immerse petri dishes in the water bath
Magnifying scope

Procedure:

Filtration

1. Collect 100mL of sample and set up sterile nitration apparatus.
2. Label petri dishes: appropriate dilutions, Q_A. (Buffer water at the beginning), Qs (blank at the end), Q_B (positive QC), and duplicate
3. Prepare desired dilutions necessary for the sample.
4. Turn on aspirator
5. Light Bunsen burner
6. To Sterilize forceps dip in alcohol and flame.
7. Open a pre-sterilized filter
8. A total volume of 100 mL's will be filtered. If necessary several volumes form each sample may have to be filtered. If sample volume is less than 20 mL add 25 mL of buffer water to the filtration funnel.
9. To obtain a homogenous sample vigorously shake the bottle.
10. Add the desired volume and rinse the funnel with no less than 25 mL of buffer water. This will prevent organism from

Colilert

B. Equipment:

Non-fluorescing sample bottles-sterilized containing sodium thiosulfate
6 watt Fluorescent UV Lamp & glasses
Incubator (35 ± 0.5°C]
Autoclave
Sealer [IDEXX Laboratories] *Figure I*
Quanti-Trays, 97-wells

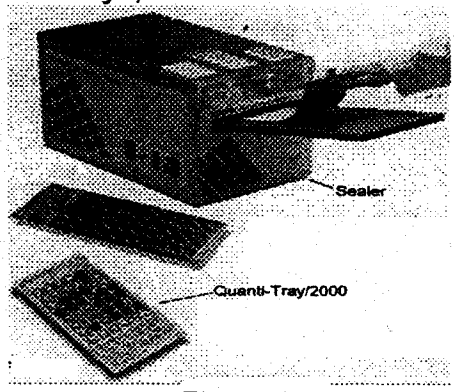


Figure I

Procedure:

QC Purchased Products:

1. Performing Quality Control on each lot of media, bottles and trays will eliminate QC on each sample run. For bottles and trays inoculate sterile water with the following cultures.

Culture	**Expected Result**
E.coli	yellow, fluorescent
Klebsiella pneumoniae	yellow, no fluorescent
Pseudomonas aeruginosa	clear, no fluorescent

m-TEC

Procedure:

Filtration

clinging to the wall and contaminating the next sample.

11. Turn off suction and remove the funnel.
12. Re-sterilize the forceps.
13. With the forceps grasp the edge of the membrane and place on the surface of the m-TEC agar plate. Allow one edge to make contact and then carefully roll the membrane onto the surface to avoid entrapment of air.
14. Close plate and secure lid.
15. Repeat steps 6-14 for all reaming samples and quality control.

Resuscitation and Incubation

1. Place petri dishes, media side-up, in a watertight baggie.
2. For a two-hour resuscitation period place the baggie, inverted in the 35.0 ± 0.5°C incubator.
3. Remove and place sample in a 44.5 ±0.5°C water bath. Invert samples and place weights on top of baggie to keep it fully immersed.

Enumeration and Identification

1. Remove plates from water bath and remove the lids. Place an absorbent pad in each lid.
2. With a pipet add about 2 ml of Urease to the pad. Make sure the pad is saturated.
3. With the forceps remove each membrane and place on the corresponding lid. Make sure the membrane is fully in contact with the absorbent pad.
4. Allow to stand for 14 to 20 minutes.
5. Use a magnifying scope and count the yellow to yellow-brown colonies.
6. Dark colonies are Urease positive, therefore not *E.coli.*
7. A countable plate is defined as one containing 20 - 80 colonies. Plates above should be designated TNTC (Too Numerous to Count), below is TFTC (To Few to Count).
8. Record the number of positive colonies and dilution for each sample.

Colilert

Procedure:

Incubation

1. Place in a 35± 0.5°C incubator for 24 hours

Enumeration and Identification

After 24 hours remove and count the blue fluorescent wells using the Fluorescent UV Lamp. A color comparator is used a comparison tool for minimum yellow color change and for fluorescence. Sample wells that are clear (no color change) are negative for total coliform. All yellow wells are counted as positive for total coliform. Then examine all total coliform positive wells for fluorescence. Blue-fluorescent wells are counted as positive for is . Record the number of positive large and small wells. Samples incubated over 28 hours use the following guideline: no yellow color present the sample is negative. A yellow color or fluorescing in VOIDED

Calculations:
1. Multiply the number of colonies counted on the plate by the dilution factor.
2.These calculated numbers represent the number of thermotolerant *E.coli* colonies/ 100ml of sample.

Calculations:
1.Use the MPN (Minimum Probable Number) table provided by IDEXX and determine the MPN for *E.coli.*
2. Find the number of small well across the top of the Table. Then go down the chart until and find the number of large wells. Where the large and small well results intercept is the MPN.
3. MPN is a number, based on certain probability formulas and is an estimate of the mean density of coliforms in the sample.

METHODOLOGY

Supplies (collection bottles and glassware), purchased media and lab prepared media underwent strict quality control. All were tested with positive and negative controls. The pre-sterilized sample bottles contained sodium thiosulfate and were marked for 100mLs..

The evaluation of m-TEC and Colilert consisted of 50 duplicate samples (100 total analyzed) collected simultaneously from White River (7) and chlorinated /dechlorinated effluent from the Anderson Wastewater Treatment Plant (43) from September 19, thru October 16, 2000. All samples consisted of 100 mL's. They were processed and incubated according to the prescribed methods. After 24 hours of incubation, m-TEC plates and Colilert were enumerated as previously described. Standard Methods requires that all results be reported as whole numbers. Samples meeting the criteria of *Standard Methods* were included in the statistical analyses.

A random, sixty-eight plates or trays (68%), portion of the sample were confirmed. A "portion" (a positive colony or a loop full from a positive well) of numerous positive samples were confirmed using m-endo Les, Eosin Methylene Agar and ENTEROTUBES™ from BBL/Difco.

RESULTS

Bacterial Identifications

Table I shows the results *for a*ll 100 samples and confirmed microorganisms. A result of 0 was considered negative, while any with a number greater than 0 was considered to be positive. A majority of the samples fell in the range of 0 to 10, while only 17 were greater than 10. All of the data was graphed using a line graph, as shown in *Graph I.* The number and type of

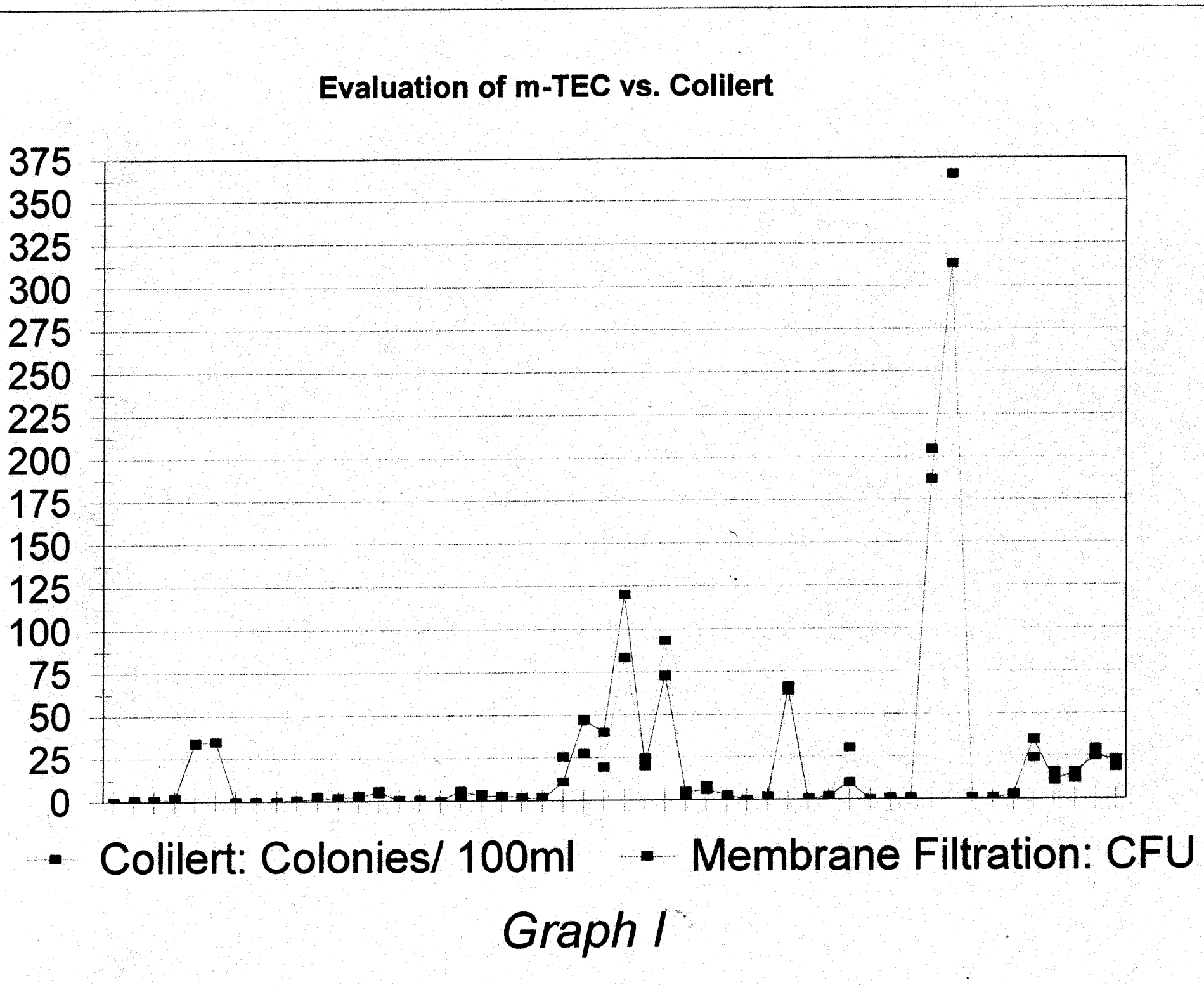
Table I

Membrane Filtration and Colilert Comparison

for

September 19 thru October 16, 2000

Sample Number	Date	Sample Identification	Colilert: Colonies/ 100ml	Membrane Filtration: CFU	Confirmation	
					Colilert	Membrane Filtration
1	09/18/00	Plant 1	0	0		
2		Plant 2	1	0	*Serratia Plymuthica*	
3		Plant 3	1	0	*E.coli*	
4	09/19/00	Plant 1	2	1	*Klebsiella Ozaenae*	*E.coli*
5		Plant 2	34.5	34	*E.coli*	*E.coli*
6		Plant 3	35	35	*no answer*	*E.coli*
7	09/20/00	Plant 1	0	0		
8		Plant 2	0	0		
9		Plant 3	0	0		
10	09/21/00	Plant 1	0	1		
11		Plant 2	3	1		
12	09/27/00	Plant 1	2	2	*E.coli*	*E.coli*
13		Plant 2	3.1	2	*Citrobacter Fruendii*	*E.coli*
14		Plant 3	5.2	6	*E.coli*	*Yersinia Frederiksenii (non ayptical) (atypical, -lysine)*
15	09/28/00	Plant 1	1	0	*Citrobacter Fruendii*	
16		Plant 2	1	0	*E.coli*	
17		Plant 3	0	0		
18	10/02/00	Plant 1	3.1	6	*E.coli*	*Yersinia enterocolitica*
19		Plant 2	4.1	3	*no answer*	*E.coli*
20		Plant 3	0	3		*E.coli*
21		Plant 4	1	2	*Yersinia enterocolitica*	*E.coli*
22		Plant 5	2	1	*Versinia Frederiksenii (non atypical) (-Lys atypical)*	*E.coli*
23		Plant 6	25.6	11	*E.coli*	*E.coli*
24	10/03/00	Veterans Bridge	27.5	47	*E.coli*	*E.coli*
25		Madison Ave. Bridge	19.5	40	*E.coli*	*E.coli*
26		500 E Bridge	83.6	120	*E.coli*	*E.coli*
27		Truman Bridge	24.6	20	*E.coli*	*E.coli*
28		600 W Bridge	93.4	73	*E.coli*	*E.coli*
29	10/03/00	Plant 1	2	5	*E.coli*	*E.coli*
30		Plant 2	8.4	6	*E.coli*	*E.coli*
31		Plant 3	2	3	*E.coli*	
32	10/04/00	Plant 1	0	0		
33		Plant 2	0	2		*E.coli*
34	10/05/00	Plant 1	66.3	64		
35	10/09/00	Plant 1	1	0	*E.coli*	
36		Plant 2	1	2	*E.coli*	*E.coli*
37		Plant 3	30.5	10	*E.coli*	*E.coli*
38	10/10/00	Plant 1	0	0		
39		Plant 2	1	0	*E.coli*	
40		Plant 3	1	1	*E.coli*	*Klebsiella Ozaenae*
41		500 E Bridge	204.6	187	*Citrobacter Fruendii*	*E.coli*
42		600 W Bridge	365.4	313.3	*E.coli*	*E.coli*
43	10/11/00	Plant 1	0	1		*E.coli*
44		Plant 2	1	0	*E.coli*	
45	10/12/00	Plant 1	0	3		*E.coli*
46		Plant 2	24	35	*Klebsiella Oxtoca*	*E.coli*
47	10/16/00	Plant 1	15.8	10.9	*E.coli*	*E.coli*
48		Plant 2	12.1	15.5	*E.coli*	*E.coli*
49		Plant 3	29.4	25	*Klebsiella pneumponiae*	*E.coli*
50		Plant 4	18.7	23	*E.coli*	*E.coli*


Evaluation of m-TEC vs. Colilert
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
Colilert: Colonies/ 100ml
Membrane Filtration: CFU

Graph I

confirmed organisms are listed in *Table II*. *E.coli* was confirmed in 69.4 percent of the wells and the other 30.6 percent confirmed as follows: Unknown organisms, *Serritia plymuthica, Klebsiella oxtoca, Yersinia enterocolitica, Citrobacter fruendii, Klebsiella pneumoniae, Yersinia frederiksenii and Klebsiella ozaenae.*

Organism Confirmed	Colilert	m-TEC
Unknown	2	
Serritia plymuthica	1	
Klebsiella oxtoca	1	
Yersima enterocolitica	1	1
Citrobacter fruendii	3	
E.coli	25	29
Klebsiella pneumoniae	1	
Yersima Frederiksenii (none atypical) (atypical, -lysine)	1	1
Klebsiella ozaenae	1	1
TOTAL	35	

Table II

The sizeable recovery of total coliforms was due to the fact that *E.coli* and fecal coliforms both grow in the Colilert media; whereas most fecal coliforms are eliminated on the m-TEC agar because they are not thermophilic and they are. From the yellow to yellow-brown colonies on m-TEC 90.6 percent were confirmed as *E.coli*, while only 66 percent were confirmed with Colilert.

Statistical Analysis

A *Linear Correlation and Regression Analysis* was conducted using Quattro Pro and VassarStats (Attachment 1) with m-TEC as the dependent *y* variable and Colilert taken as the independent *x* variable. The results are shown below: The closer the result, r^2, is to +1 or –1 the "samples are equivalent". A graphical representation is shown in Graph II.

	Quattro Pro	**VassarStats**
Regression	*Calculating y*	*Calculating y*
Constant/Intercept	1.85	1.87
Standard Error of *y* estimation	9.158	9.156
r	0.99	0.99
r Squared	0.97	0.97
No. of Observations	50	50
Slope	0.88	0.88

VassarStats: Linear Correlation and Regression

➢r = the Pearson product-moment correlation coefficient;
➢r^2 = the coefficient of determination;
➢the slope of the regression line;
➢the Y intercept of the regression line;
➢the standard error of estimate; and
➢the value of t associated with the calculated value of r.

Values entered:

Pairs	X	Y	XY	Pairs	X	Y	XY
1	0	0	0	26	83.6	120	10032
2	1	0	0	27	24.6	20	492
3	1	0	0	28	93.4	73	6818.2
4	2	1	2	29	2	5	10
5	34	34	1173.5	30	8.4	6	50.4
6	35	35	1225	31	2	3	6
7	0	0	0	32	0	0	0
8	0	0	0	33	0	2	0
9	0	0	0	34	66.3	64	4243.2
10	0	1	0	35	1	0	0
11	3	1	3	36	1	2	2
12	1	2	2	37	30.5	10	305
13	3.1	2	6.2	38	0	2	0
14	5.2	6	31.2	39	1	0	0
15	1	0	0	40	1	1	1
16	1	0	0	41	204.5	183	8260.27
17	0	0	0	42	365.4	313.3	114479.8
18	3.1	6	18.6	43	0	1	0
19	4.1	3	12.3	44	1	0	0
20	0	3	0	45	0	30	0
21	1	2	2	46	24	35	840
22	2	1	2	47	5.3	10.9	172.2
23	25.6	11	281.6	48	12.2	15.5	189.1
24	27.5	47	1292.5	49	29.4	25	735
25	19.5	40	780	50	16.7	23	430.1

Summary values:

Values	X	Y	XY
n	50		
sum	1157.4	1114.7	181898.09
mean	23.148	22.294	
sum_sq	203587.78	166694.95	
SS	176796.2848	141843.8282	156095.0144
variance	3608.0874	2894.772	
st. dev.	60.0674	53.8031	

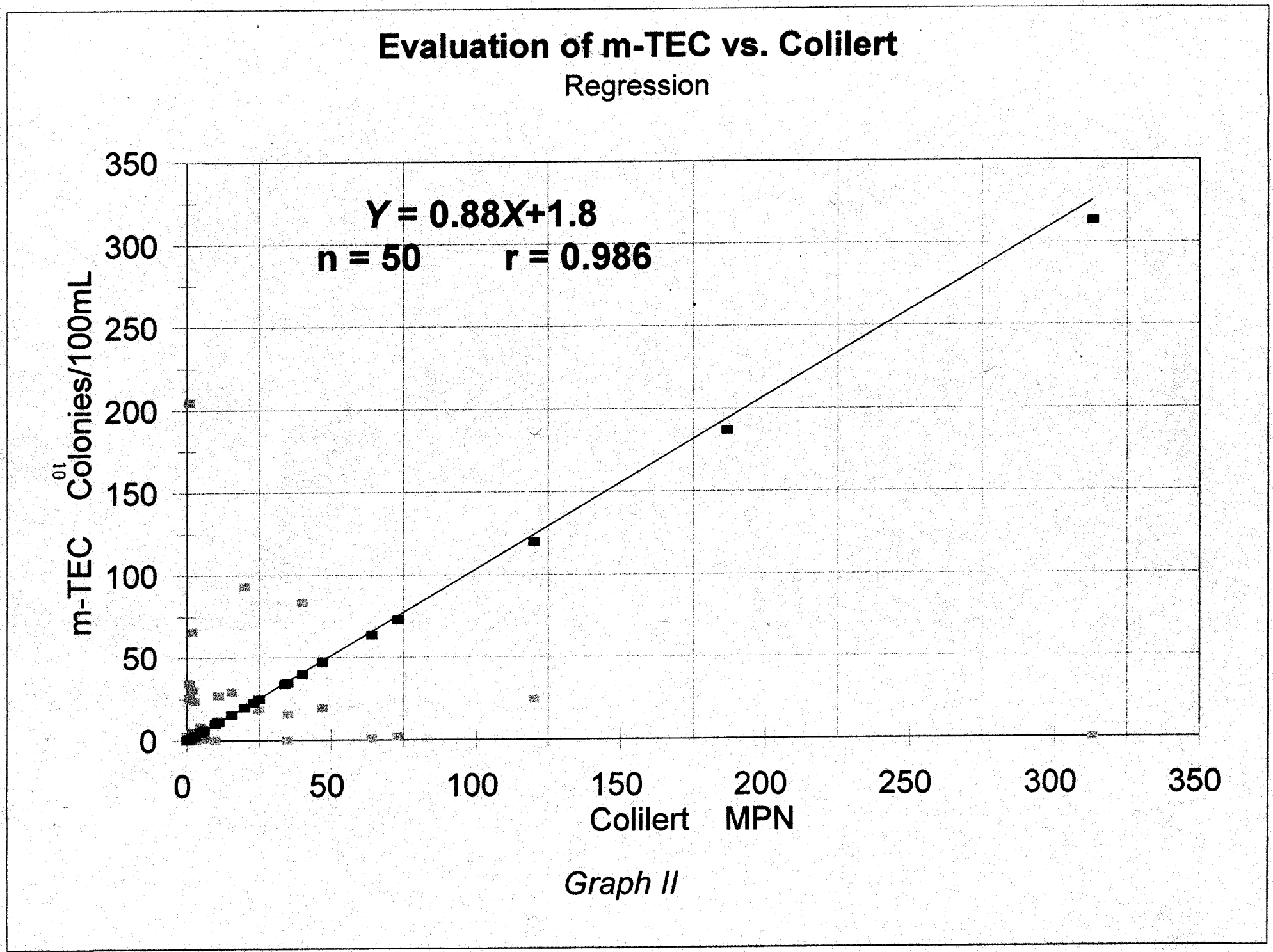
Variances and standard deviations are calculated with denominator = n-1.

r	r^2	Slope	Intercept	Standard Error
0.986	0.972	0.883	1.8564	9.1585

t	df
40.535	48

P		
	one-tailed	2.5
	two-tailed	5.000000

Attachment I


Evaluation of m-TEC vs. Colilert
Regression
Y = 0.88X+1.8
n = 50 r = 0.986
m-TEC Colonies/100mL
10
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
Colilert MPN

Graph II

The regression analyses showed that the two analytical methods resulted in equivalent results, with an r^2 of 0.97.

The *Student's t Test for Correlated Samples* was conducted comparing the mean value of the Colilert method *E.coli* count and the mean value of m-TEC method *E.coli* count. The results are contained in Attachment II.

Calculated: $t = t_{obs}$ = +.53 degrees of freedom

Sample number of pairs = 50 - 1 = 49

The critical value for t for 49 degrees of freedom is 2.01 at a 95% confidence level

Thus we can say that the results of the two-tailed test show $-t<t_{obs}<+t$ (-2.01<.53<+2.01). The critical value of -2.01 for 49 degrees of freedom at a 95% confidence level is less than the observed t value of 0.53 and the observed t value of 0.53 is less than the critical value of +2.01 for 49 degrees of freedom at a 95% confidence level. These results prove that a 95% confidence level, there is no significant difference between the mean of the results of the Colilert samples and the mean of the results of the m-TEC samples.

The above data and Graph *I* show that both methods parallel each other.

DISCUSSION

Bacterial counts can be reported as Colony Forming Units (CFU), colonies/100 mL and Most Probable Number (MPN. CFU is used to designate the type of method used. A report would include the method, incubation temperature, time and media. Below are examples for m-TEC and Colilert.

➢m-TEC would be reported as:

CFU/mL, membrane filtration, 44°C2h, 35°C/22h, m-TEC

➢Colilert would be reported as:

CFU/mL, Chromogenic Substrate Test, 35°C/24h, Colilert

Results are reported as colonies /100mL are an estimate of bacterial density. It should be noted that bacterial counts do not follow the Poisson distribution and are not distributed randomly therefore, indicating that colony counts are not absolute numbers. Colonies/100mL are associated with methods utilizing various types of agars. The bacterial density is calculated by multiplying the number of colonies counted by 100 then divided by milliliters of sample filtered or

VassarStats Printable Report: t-Test for Correlated Samples

Values Entered

Sample A	Sample B	Sample A	Sample B
0	0	83.6	120
1	0	24.6	20
1	0	93.4	73
2	1	2	5
34.5	34	8.4	6
35	35	2	3
0	0	0	0
0	0	0	2
0	0	66.3	64
0	1	1	0
3	1	1	2
2	2	30.5	10
3.1	2	0	0
5.2	6	1	0
1	0	1	1
1	0	204.6	187
0	0	365.4	313.3
3.1	6	0	1
4.1	3	1	0
0	3	0	3
1	2	24	35
2	1	15.8	10.9
25.6	11	12.1	15.5
27.5	47	29.4	25
19.5	40	18.7	23

Summary Data

	A	B	Total
n	50	50	100
- X	1157.4	1114.7	2272.1000000000003
- X^2	203587.77999999997	166694.95	370282.73
SS	176796.2848	141843.8282	318658.3459
mean	23.148	22.294	22.721

$Mean_A$—$Mean_B$	t	df
0.854	+0.53	49

P		
	one-tailed	0.2992525
	two-tailed	0.598505

E.coli/100mL = Number of *E.coli* colonies counted x 100

mL sample filtered

Most Probable Number (MPN) uses a statistical model referred to as the MPN Index to determine the bacterial density. MPN is associated with a "liquid medium" and is calculated by counting the number of positive tubes and relating this number to the MPN Index.

Sixty-nine percent of the yellow and fluorescent wells were confirmed as *E.coli*. The poor percent recovery was due to the fact the Colilert, unlike m-TEC, is a liquid medium and it does not eliminate existing coliform bacteria. Isolation of *E. coli* using m-TEC, involves removing a single yellow or yellow-brown colony and while Colilert involves removing a loop full of the positive media. That loop full may or may not contain *E.coli*. Colilert is designed to confirm *E.coli* and Fecal Coliforms; therefore, it should not have to be reconfirmed.

The statistical analysis of m-TEC and Colilert demonstrate that they are comparable. The graphical illustration (Graph I) demonstrated that the data from both m-TEC and Colilert paralleled each other. This is further confirmed by the *Linear Regression* analysis of r^2 of 0.97 and a *Student's t Test for Correlated Samples* of a +.53.

The side-by-side comparison of the two procedures showed that Colilert is a faster and less involved method than the m-TEC method. Specifically Colilert offers many advantages over m-TEC.

1) In 24 hours a 100 mL volume of wastewater can specifically identify and confirm *E.coli* without any transfers.
2) Less time in the preparation and analysis of samples
3) Quality control performed on item lots, not for every run, which saves in supplies and time.
4) Colilert uses purchased media, resulting in no preparation time and no errors in media preparation.
5) Considerably less subjectivity in interpreting results with Colilert than with m-TEC.
6.) Heterotrophic bacterial interference is eliminated, because they do not react with the nutrients in the media, therefore giving no reaction.
7.) Samples that contain solids are easily analyzed, whereas with m-TEC the samples would be difficult to filter.
8.) Confirmation of results from Colilert is not necessary because of the specific reactions of Total Coliforms and *E.coli*.

The above information and the statistical analyses demonstrate that the Colilert Method can be substituted for m-TEC. It is the opinion of the author that Colilert is the method of choice.